Selecting High Quality Forage Legumes

Improving Pest Resistance and Quality While Maintaining Yield and Persistence

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Content

Introduction

High Yield! Disease and Insect Resistance! High Quality! Tolerance to Frequent Cutting! Drought Tolerance! Winterhardy! Persistence! What a demand? – But yet the consumer is demanding all of this from our forage legumes. Annual crops such as corn, soybeans, oats, and wheat are never subjected to all these extreme criteria. Can perennial legume cultivars be developed to fill all these demands??? Farmers are looking for all these attributes as they choose the legume cultivar to plant. Once established, yield, longevity, quality and to a certain extent, pest resistance, are a function of management. Probably the two most desirable attributes of a forage legume are persistence and consistent high yields. These attributes are not always positively associated, and they are quite often negatively associated with high quality forage. What is persistence? I like to think of it as stand longevity and to describe it as the survival of plant material against specific stresses unique to the existing environment. Forage stand depletion begins as the seed is planted and becomes evident as soon as harvesting, grazing or cutting commences. The loss of stand continues progressively, depending on the interaction of climatic, pest, edaphaic, management, and physiological factors as well as competition among species. Plants' responses to these factors and their ability to persist under hay or grazing conditions is dependent upon the degree of stress imposed. In the field, the environment is continually changing, imposing an ever changing set of

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stress conditions. Improving persistence is probably the most cost effective activity a plant breeder can undertake to improve forage legumes.

Recurrent Selection For Persistence

First let us establish a basic difference between most annual grain or soybean crops and the perennial forages, especially the legumes. As the consumer or farmer sees them, the former are generally pure line or hybrids, i.e., very uniform in their genetic constitution. In contrast, most forages are cross pollinated species and each cultivar or variety is made up of numerous genetically different individuals. In addition, many forage species are polyploid, have more than two (diploid) copies of each chromosome, which makes selection and genetic manipulation of populations much more difficult. Thus, breeding procedures such as pure line development, hybrids, etc. are of limited value to the forage breeder.

Probably the most frequently used breeding procedure has been to select plants based on their visual appearance, response to stress or chemical constitution (phenotypes). Basically, the plants are evaluated for the appropriate attribute (disease resistance, protein, yield, etc.) and, according to their response, the most desirable plants are selected and intercrossed to produce progeny for subsequent evaluation and selection. If the attribute is highly heritable and controlled by a single or few genes, then the desired level of performance is achieved quite rapidly (one or two cycles of selection). On the other hand, if the attributes have low heritability and are controlled by many genes, then selection progress is slow and many cycles of selection are required to achieve the desired level of response. Unfortunately, most of the attributes of a good cultivar, especially yield, quality and persistence, have low heritability and are controlled by many genes.

It is quite easy to effectively select for some attributes such as pest resistance, quality parameters or seed production on an annual basis, usually under controlled conditions, and complete many cycles of selection in a relatively short period of time. This was the procedure used early in our program on the improvement of red clover (Smith and Kretchmer, 1989), but I became concerned as to what effect selection, on an annual basis, has on yield and persistence. Therefore, we evaluated in the field, four populations that represented four annual cycles of selection conducted in the greenhouse for resistance to the disease northern anthracnose. We measured forage yield, the genetic variation for yield and persistence of these four populations in comparison to the original base population. Both forage yield and the genetic variance for yield increased in the first cycles but steadily decreased after the second and first cycles of selection, respectively (Figs. 1A and 1B). In contrast, persistence did not change significantly over the same cycles

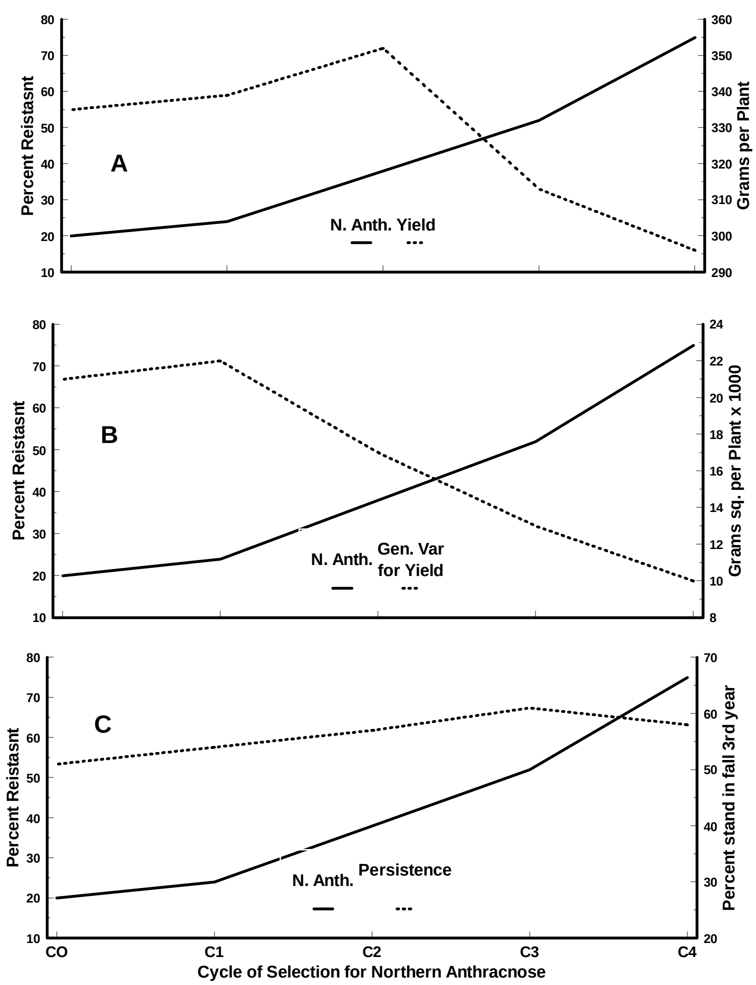

Figure 1. Response of annual selection for northern anthracnose in red clover on yield (A), genetic variance for yield (B) and persistence (C).

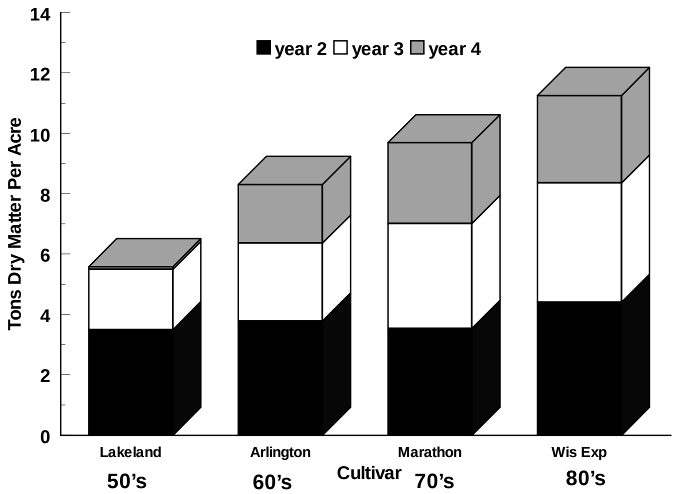

Figure 2. Progressive improvement in the performance of red clover resulting from four decades of breeding for persistence and disease resistance.

"PECTIN - A new quality parameter?"

of selection (Fig. 1C). Even though persistence was not changed during annual selection for disease resistance, we believe it appropriate to field test the selections after two or three cycles of annual selection. This provides an opportunity to evaluate the germplasm for agronomic performance such as persistence and forage yield. If conditions are appropriate, it also provides opportunities to evaluate the selected populations for field tolerance to the desired attribute. The improved germplasm in these field tests also provide source material for selection for persistence after several years of evaluation.

With this in mind and in an attempt to develop persistent red clover germplasm, the emphasis of the USDA-ARS and University of Wisconsin breeding program has been to select 70 to 100 surviving, reasonably healthy plants from 3- or 4-year old field tests and to intercross these selected plants, thus, generating a new cycle of persistent selection. The subsequent progeny are again subjected to further attribute evaluation and screening. Since the 1950's this process has been repeated four times. Progress from this process is presented in Figure 2. Substantial improvement in forage dry matter yield at the end of the 4th year has been achieved. In the past, red clover stands declined rapidly between the 2nd and 3rd years, often severe enough to discontinue the sward. This problem was somewhat reduced by the release of Arlington (Smith et al. 1973) and Marathon (Smith 1994). An added bonus is that resistance to *Fusarium* root rot increased (without imposed selection) from 39% in Lakeland (released in 1953) to 65% in the new Wisconsin Experimentals. Undoubtedly, selection for persistence in 3- or 4-year-old stands was also selecting for resistance to *Fusarium* (Smith et al. 1995). Currently, were are continuing to select (Wis. Exp., Fig. 2) within these cultivars to improve pest resistance using a combination of recurrent phenotypic selection, progeny testing and family selection.

Forage Quality vs. Persistence

Forage legume species have excellent forage quality when harvested during the early phases of reproductive growth. However, as plant breeders have attempted to alter the less digestible fraction (lignin-carbohydrate complexes) of the plant they have generally met with undesirable correlated responses in other attributes. Kephart et al. (1989) reported that the leaf-to-stem ratio was higher in low lignin lines than high lignin lines selected by Hill (1981) for whole plant acid detergent lignin (ADL) concentration. Significant differences observed in quality parameters of leaf and stem cell wall fractions of these divergent lines were generally diluted by the differences in leaf-to-stem ratio between the high and low lignin populations (Kephart et al. 1990). After three years of growth in the field only 34% of the plants in the low lignin lines had survived in contrast to 64% in the high lignin lines (R.R. Smith and D.R. Buxton, unpublished data). This type of evidence is supportive of the concept that during selection for quality both the leaf and stem fractions need to be considered and that altering quality factors may have impact on persistence and yield.

PECTIN - A new quality parameter?

Estimating Pectin by NIRS

Earlier in this conference Dr. Hatfield related to you the importance of pectic polysaccharides to the cell wall matrix

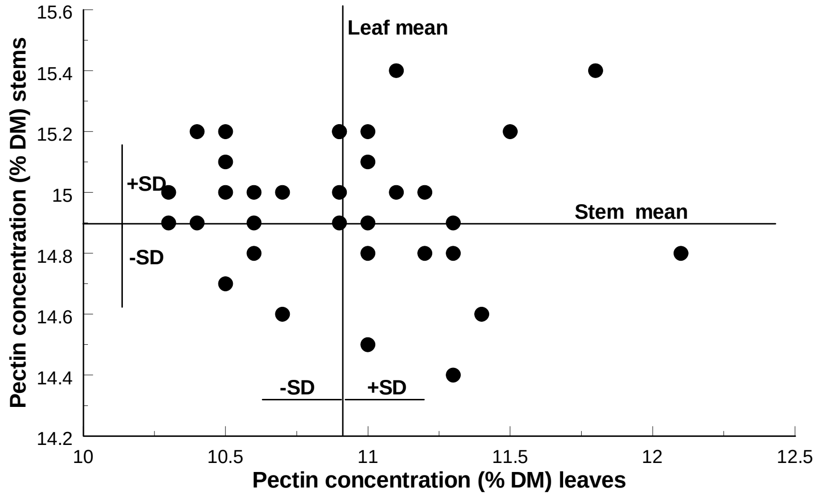

Figure 3. Distribution of 33 alfalfa cultivars or experimental lines at first harvest for pectin concentration of leaf and stem cell walls, Arlington, WI.

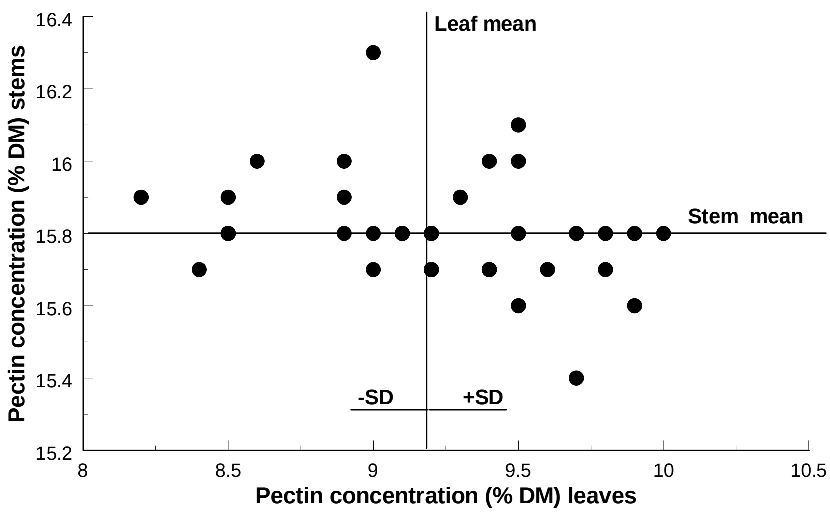

Figure 4. Distribution of 36 alfalfa cultivars or experimental lines at first harvest for pectin concentration of leaf and stem cell walls, Marshfield, WI.

and their implication to forage quality and animal nutrition. In addition, he presented data which suggested that perhaps there was sufficient genetic variation within our forage legumes to warrant further investigations. Since the process described by Dr. Hatfield is rather tedious and time consuming, we were interested in utilizing some system or technique to simplify the measurement of cell wall pectin. Using the chemical information provided by Dr. Hatfield, we generated prediction equations using near infrared reflectance spectroscopy (NIRS) spectral data of the plant tissue. In cooperation with Mr. Markus Daepp (pre-doctoral student from Switzerland), we applied the equations to the plant tissue derived from the first seasonal growth (bud stage) of alfalfa cultivars and germplasm lines (entries) being tested in the Wisconsin alfalfa variety trials at Arlington and Marshfield Research Stations under the supervision of Dr. D.J. Undersander, Wisconsin Forage Extension Agronomist. Significant differences for leaf and stem cell wall pectin concentration were observed among the 33 entries tested at Arlington, WI (Fig. 3) and for leaf cell wall pectin concentration among the 36 entries tested at Marshfield, WI (Fig. 4). It seems the most ideal germplasm would be that which is high in both leaf and stem cell wall pectin. This preliminary data from the Arlington location (Fig. 3) would suggest that at least two entries would fall into the desirable class of high leaf and stem pectin. Further analysis needs to be completed to validate these results and to expand the number of cultivars and locations tested.

Selection for Cell Wall Pectin

To further test the effectiveness of these equations, we predicted the pectin concentration of leaf and stem cell walls of 64 alfalfa plants previously selected for winter survival. From these predictions, five groups were formed - high and low leaf pectin, high and low stem pectin, and high leaf and stem pectin. Three to four plants representing each group were intercrossed. The subsequent polycross progeny from these crosses and the respective parents have been established in the field and will be evaluated for cell wall pectin concentration. The objective is to develop populations with high and low leaf pectin and high and low stem pectin to validate the effect of altered cell wall pectin concentration on animal nutrition. These polycross progeny will also be evaluated for ADF, NDF, ADL, protein, digestibility, leaf-to-stem ratio, etc. to determine correlated responses as a result of selection for cell wall pectin.

Forage Disease vs. Persistence and Yield

Diseases are a major cause of premature stand decline in forage legumes. Rarely do they act independently but merely as one component of a group of biotic and abiotic agents which interact to shorten stand life (Leath 1989). However, considerable evidence is available which suggests that, when forage disease epiphytotics occur, both yield and persistence are increased when resistance to the causal plant pathogens has been incorporated into forage legume germplasm (Barnes et al. 1969, Elgin et al. 1981, Leath 1989, Leath et al. 1996). Some diseases, especially foliar diseases, have been reported to have a negative impact on forage quality (Smith and Maxwell 1971, Leath et al. 1978, Mainer and Leath 1978, Leath 1989, Lenssen et al.1991). Therefore, I don't think there is any question as to the importance of disease resistant germplasm to forage yield and stand longevity. This relationship relative to the longevity of red clover was pointed out earlier in this paper.

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The soilborne fungus, *Mycoleptodiscus terrestris,* was recovered from decaying roots and stems of birdsfoot trefoil plants sampled from two-year-old yield trials, in 1994 and establishment year stands, in 1995 at the Arlington Agricultural Research Station, Arlington, WI. Although recognized in states south of Wisconsin, *M. terrestris* has not been implicated in poor health of forage legumes in Wisconsin. *M. terrestris* has been previously reported to be pathogenic on alfalfa, red clover and birdsfoot trefoil in Illinois (Gerdemann 1954). Presence of this pathogen in the upper Northcentral states may explain some stand establishment failures that otherwise have been undetermined. The fungus has been reported to be pathogenic on birdsfoot trefoil in Missouri (Pettit, et al. 1969) and eastern US (Carroll and Whittington 1991). Only the trefoil cultivar Dawn (Beuselinck 1994) and the germplasm CAD (Beuselinck and Steiner 1994) have been reported to have some degree of resistance (tolerance) to *M. terrestris.* However, no resistance has been identified in trefoil germplasm adapted to the northern area of the midwest. Forage legume germplasm has not been characterized extensively for reaction to *M. terrestris.*

In cooperation with Dr. C.R. Grau, we have established that the Wisconsin-derived isolates of *M. terrestris* were pathogenic on alsike, berseem, kura, red and white clover, alfalfa, birdsfoot trefoil and sweetclover. Inoculation procedures are being standardized and selection for resistance has been initiated in birdsfoot trefoil and red clover.

The Question Still Remains:

Can perennial legume cultivars be developed to fill all the demands we are imposing on it???

I believe we can improve our present germplasm to incorporate the desirable attributes that we wish to be included, but we may have to make some compromises along the way. I do not see any "quick fixes" for these improvements, especially if we are demanding longevity in the respective species. As new and expanding technologies are developed, improvement in our plant performance will require a concentrated approach involving personnel from different disciplines. With declining fiscal resources in the public sector it is imperative that we have cooperative efforts between the private and public plant breeders to maintain the level of research and germplasm improvement we currently have.

Germplasm Available for Release

Red Clover:
- Multiple disease resistant germplasm:
- Persistent, disease resistant germplasm or cultivars

Birdsfoot Trefoil:
- High yielding, large seeded, excellent seedling vigor cultivar, WITT
- High yielding, excellent seedling vigor germplasm tested as TREVIG

Kura Clover:
- High yielding, excellent seedling vigor, persistent germplasm or cultivar, WISAMB
- Large seeded populations at the diploid, tetraploid, and hexaploid level, 2xLS, 4xLS, 6xLS

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